

WHAT IS CLAIMED IS:

1. A visually detectable biomolecule of formula $B-(L-(D)_m)_n$, wherein

m and n are each an integer from one to about 5;

B is a biomolecule;

- 5 L, at each occurrence, is a spacer group comprising from one to about 10 linear atoms,

where L is attached to B by means of an ester, amide, phosphate, phosphorothioate, phosphonate, thioester, or disulfide linkage, and where the remaining linear atoms in L are selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

D, at each occurrence, is a radical of a photostable visible dye, wherein each P has one and only one linkage to a biomolecule, provided that D is not unsubstituted perylenyl.

2. A visually detectable biomolecule of formula $B-(L-(P)_m)_n$, wherein

m and n are each an integer from one to about 5;

B is a biomolecule;

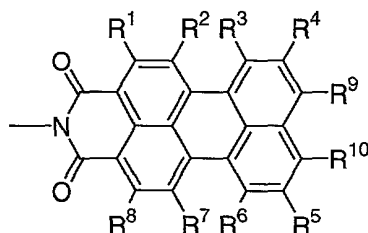
L, at each occurrence, is a spacer group comprising from one to about 10 linear atoms,

where L is attached to B by means of an ester, amide, phosphate, phosphorothioate, phosphonate, thioester, or disulfide linkage, and where the remaining linear atoms in L are selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

P, at each occurrence, is a radical of a perylene, anthracene, naphthalene, or pyrene derivative, wherein each P has one and only one linkage to a biomolecule.

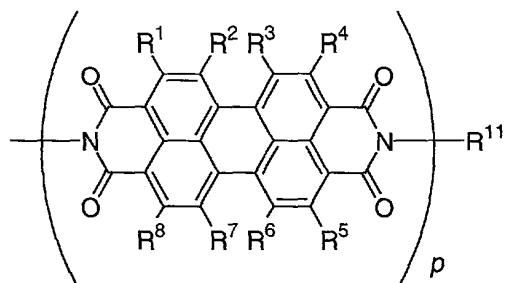
3. The visually detectable biomolecule of claim 1, wherein B is selected from the group consisting of nucleic acids, carbohydrates, amino acids, and polypeptides.
4. The visually detectable biomolecule of claim 1, wherein B is a nucleic acid.
5. The visually detectable biomolecule of claim 1, wherein B is selected from the group consisting of enzymes, receptors, receptor ligands, antibodies, and glycoproteins.
6. The visually detectable biomolecule of claim 2, wherein P is a radical of a perylene derivative.
7. The visually detectable biomolecule of claim 6, wherein the perylene derivative is a perylene imide, perylene bisimide, or perylene hydrazamimide.
8. The visually detectable biomolecule of claim 7, wherein P has the formula:



wherein

$R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9$, and R^{10} are radicals independently selected from the group consisting of hydrogen, halogen, cyano, hydroxy, alkoxy, amino, alkylamino, dialkylamino, heterocyclic radical, alkyl, and aryl, wherein the alkyl and aryl groups may be optionally substituted, and wherein adjacent radicals can form a carbocyclic or heterocyclic ring.

9. The visually detectable biomolecule of claim 8, wherein P has the formula:

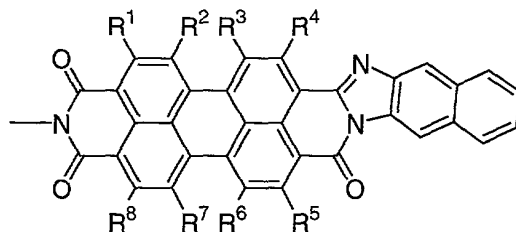


wherein p is 0, 1, or 2;

$R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9$, and R^{10} are radicals independently selected from the group consisting of hydrogen, halogen, cyano, hydroxy, alkoxy, amino, alkylamino, dialkylamino, heterocyclic radical, alkyl, and aryl, wherein the alkyl and aryl groups may be optionally substituted, and wherein adjacent radicals can form a carbocyclic or heterocyclic ring; and

R^{11} is C_1-C_6 alkyl, C_6-C_{10} aryl, or $(C_6-C_{10})ar(C_1-C_6)alkyl$.

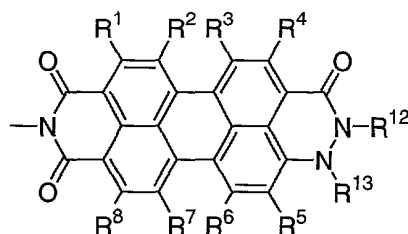
10. The visually detectable biomolecule of claim 8, wherein P has the formula:



wherein

$R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9$, and R^{10} are radicals independently selected from the group consisting of hydrogen, halogen, cyano, hydroxy, alkoxy, amino, alkylamino, dialkylamino, heterocyclic radical, alkyl, and aryl, wherein the alkyl and aryl groups may be optionally substituted, and wherein adjacent radicals can form a carbocyclic or heterocyclic ring.

11. The visually detectable biomolecule of claim 7, wherein **P** has the formula:



wherein

$R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9$, and R^{10} are radicals independently selected from the group consisting of hydrogen, halogen, cyano, hydroxy, alkoxy, amino, alkylamino, dialkylamino, heterocyclic radical, alkyl, and aryl, wherein the alkyl and aryl groups may be optionally substituted, and wherein adjacent radicals can form a carbocyclic or heterocyclic ring; and

R^{12} and R^{13} are independently C_1-C_6 alkyl, C_6-C_{10} aryl, or $(C_6-C_{10})ar(C_1-C_6)$ alkyl.

12. A visually detectable biomolecule standard of formula $B-(-L-(D)_m)_n$, wherein m and n are each an integer from one to about 5;

B is a biomolecule;

L is a spacer group comprising from one to about 10 linear atoms,

where **L** is attached to **B** by means of an ester, amide, phosphate,

phosphorothioate, phosphonate, thioester, or disulfide linkage, and where

the remaining linear atoms in L are selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

5 D, at each occurrence, is a radical of a photostable visible dye, wherein each D has one and only one linkage to a biomolecule, provided that D is not unsubstituted perylenyl;

wherein the standard has a defined molecular weight.

13. A visually detectable biomolecule standard of formula $B-(L-(P)_m)_n$, wherein m and n are each an integer from one to about 5;

B is a biomolecule;

L is a spacer group comprising from one to about 10 linear atoms,

where L is attached to B by means of an ester, amide, phosphate, phosphorothioate, phosphonate, thioester, or disulfide linkage, and where the remaining linear atoms in L are selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

20 P, at each occurrence, is a radical of a perylene, anthracene, naphthalene, or pyrene derivative, wherein each P has one and only one linkage to a biomolecule;

wherein the standard has a defined molecular weight.

14. A kit for determining the size of a test biomolecule, comprising a collection of two or more visually detectable biomolecule standards of formula $B-(L-(D)_m)_n$, wherein
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m and n are each an integer from one to about 5;

B is a biomolecule;

L is a spacer group comprising from one to about 10 linear atoms,

where L is attached to B by means of an ester, amide, phosphate,
phosphorothioate, phosphonate, thioester, or disulfide linkage, and where
the remaining linear atoms in L are selected from the group consisting of
carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally
can be included in a ring; and

D, at each occurrence, is a radical of a photostable visible dye, wherein each D
has one and only one linkage to a biomolecule;

wherein each standard has a defined molecular weight.

15. A kit for determining the size of a test biomolecule, comprising a collection of
two or more visually detectable biomolecule standards of formula $B-(L-(P)_m)_n$,
wherein

m and n are each an integer from one to about 5;

B is a biomolecule;

L is a spacer group comprising from one to about 10 linear atoms,

where L is attached to B by means of an ester, amide, phosphate,
phosphorothioate, phosphonate, thioester, or disulfide linkage, and where
the remaining linear atoms in L are selected from the group consisting of
carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally
can be included in a ring; and

P, at each occurrence, is a radical of a perylene, anthracene, naphthalene, or pyrene derivative, wherein each P has one and only one linkage to a biomolecule;

wherein each standard has a defined molecular weight.

- 5 16. A method for determining the size of a test biomolecule, comprising:
- (a) subjecting the visually detectable biomolecule kit according to claim 14 or 15 to conditions under which the biomolecule standards migrate to different positions according to molecular weight, thereby producing a visual ladder of biomolecule standards;
- (b) subjecting the test biomolecule to the same conditions employed in step (a); and
- (c) comparing the migration of test biomolecule to the visual ladder of biomolecule standards to determine the molecular weight of the test biomolecule.

- 10 17. A method for visually detecting a biomolecule, comprising
- (a) providing a biological system with a visually detectable biomolecule of formula $B-(L-(D))_m$, wherein
- m and n are each an integer from one to about 5;
- B is a biomolecule;

20 L is a spacer group comprising from one to about 10 linear atoms,

where L is attached to B by means of an ester, amide, phosphate, phosphorothioate, phosphonate, thioester, or disulfide linkage, and where the remaining linear atoms in L are selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

D, at each occurrence, is a radical of a photostable visible dye, wherein each D has one and only one linkage to a biomolecule, provided that D is not unsubstituted perylenyl; and

(b) detecting the biomolecule by its visible properties.

18. A method for visually detecting a biomolecule, comprising

(a) providing a biological system with a visually detectable biomolecule of formula $B-(L-(P)_m)_n$, wherein

m and n are each an integer from one to about 5;

B is a biomolecule;

L is a spacer group comprising from one to about 10 linear atoms,

where L is attached to B by means of an ester, amide, phosphate, phosphorothioate, phosphonate, thioester, or disulfide linkage, and where the remaining linear atoms in L are selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

P, at each occurrence, is a radical of a perylene, anthracene, naphthalene, or pyrene derivative, wherein each P has one and only one linkage to a biomolecule; and

(b) detecting the biomolecule by its visible properties.

19. A method for visually detecting a biomolecule, comprising:

(a) contacting a biomolecule with a reactive dye of $(D)_n-L-X$, wherein

D is a radical of a photostable visible dye, provided that D is not unsubstituted perylenyl;

L is a spacer group comprising from one to about 10 linear atoms selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

5 wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

X is a reactive group that enables attachment to an amino, hydroxy, carboxyl, or sulfhydryl group on a biomolecule;

whereby a visually detectable biomolecule of formula $B-(L-(D)_m)_n$ is produced; and

(b) detecting the biomolecule by its visible properties.

20. A method for visually detecting a biomolecule, comprising:

(a) contacting a biomolecule with a reactive dye of formula $(P)_n-L-X$, wherein P is a radical of a perylene, anthracene, naphthalene, or pyrene derivative; L is a spacer group comprising from one to about 10 linear atoms selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

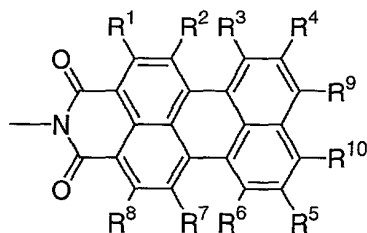
wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

X is a reactive group that enables attachment to an amino, hydroxy, carboxyl, or sulfhydryl group on a biomolecule;

whereby a visually detectable biomolecule of formula $B-(L-(P)_m)_n$ is produced; and

(b) detecting the biomolecule by its visible properties.

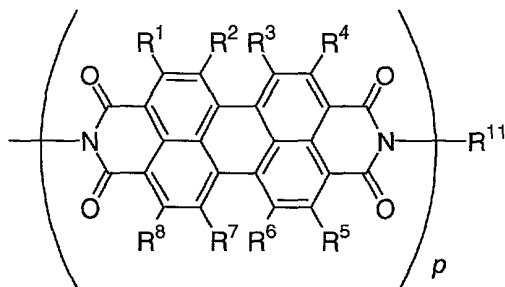
21. The method of claim 19, wherein **B** is selected from the group consisting of nucleic acids, carbohydrates, amino acids, and polypeptides.
22. The method of claim 19, wherein **B** is a nucleic acid.
23. The method of claim 19, wherein **B** is selected from the group consisting of enzymes, receptors, receptor ligands, antibodies, and glycoproteins.
24. The method of claim 19, wherein the perylene derivative is a perylene imide, perylene bisimide, or perylene hydrazamimide.
25. The method of claim 24, wherein **P** has the formula:



wherein

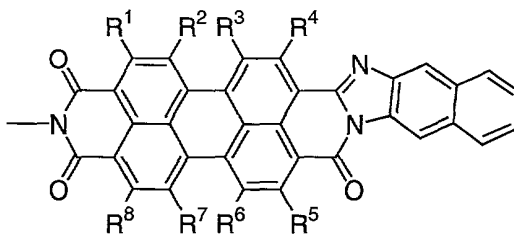
R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , and R^{10} are radicals independently selected from the group consisting of hydrogen, halogen, cyano, hydroxy, alkoxy, amino, alkylamino, dialkylamino, heterocyclic radical, alkyl, and aryl, wherein the alkyl and aryl groups may be optionally substituted, and wherein adjacent radicals can form a carbocyclic or heterocyclic ring.

26. The method of claim 24, wherein **P** has the formula:



wherein p is 0, 1, or 2; and R^{11} is C_1 - C_6 alkyl, C_6 - C_{10} aryl, or $(C_6$ - $C_{10})$ ar(C_1 - C_6)alkyl.

27. The method of claim 24, wherein P has the formula:



28. A reactive dye of formula $(D)_n$ - L - X , wherein

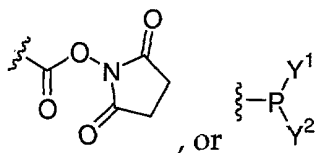
n is an integer from 1 to about 5;

D is a radical of a photostable visible dye, provided that D is not unsubstituted perylenyl;

L is a spacer group comprising from one to about 10 linear atoms selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

X is



wherein Y^1 and Y^2 are independently dialkylamino, *N*-heterocyclic radical, or OZ, where Z is a protecting group.

29. A reactive dye of formula $(P)_n-L-X$, wherein

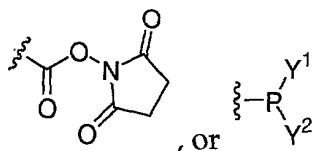
n is an integer from 1 to about 5;

5 P is a radical of a perylene, anthracene, naphthalene, or pyrene derivative;

L is a spacer group comprising from one to about 10 linear atoms selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

X is

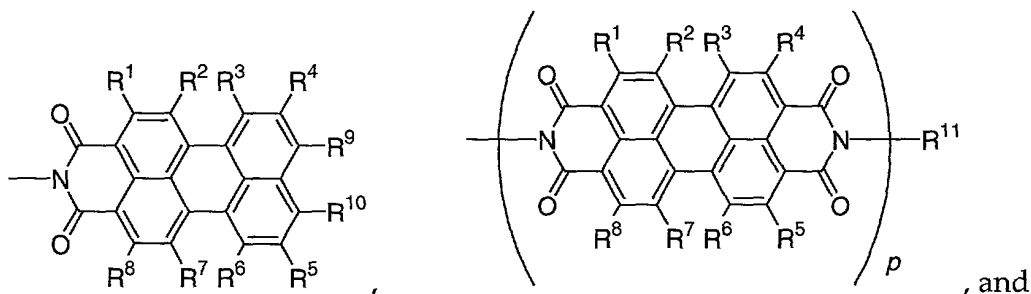


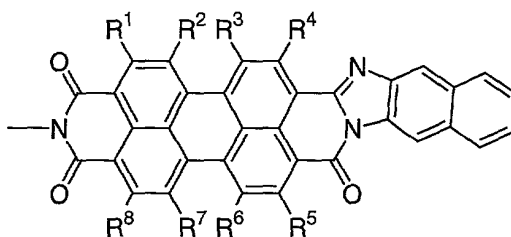
wherein Y^1 and Y^2 are independently dialkylamino, *N*-heterocyclic radical, or OZ, where Z is a protecting group.

30. A reactive dye of formula $(P)_n-L-X$, wherein

15 n is an integer from 1 to about 5;

P is a radical of a perylene derivative having a formula selected from the group consisting of:





wherein

$R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9$, and R^{10} are radicals independently selected from the group consisting of hydrogen, halogen, cyano, hydroxy, alkoxy, amino, alkylamino, dialkylamino, heterocyclic radical, alkyl, and aryl, wherein the alkyl and aryl groups may be optionally substituted, and wherein adjacent radicals can form a carbocyclic or heterocyclic ring;

p is 0, 1, or 2;

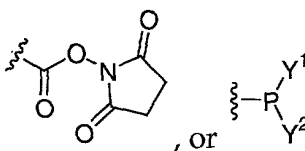
R^{11} is C_1-C_6 alkyl, C_6-C_{10} aryl, or $(C_6-C_{10})ar(C_1-C_6)alkyl$;

L is a spacer group comprising from one to about 10 linear atoms selected from the group consisting of carbon, oxygen, nitrogen, and sulfur;

wherein the linear atoms in L can be optionally substituted and optionally can be included in a ring; and

X is a reactive group that enables attachment to an amino, hydroxy, carboxyl, or sulfhydryl group on a biomolecule.

31. The reactive dye of claim 30, wherein X has the formula:



wherein Y^1 and Y^2 are independently dialkylamino, N -heterocyclic radical, or OZ , where Z is a protecting group.

32. The reactive dye of claim 29 or 31, wherein Z is alkyl, allyl, aryl, or cyanoalkyl.
33. The reactive dye of claim 29 or 31, wherein Y¹ is cyanoethyl and Y² is diisopropylamino.

Approved for filing